IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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For:	Taxoid-Fatty Acid Conjugates and Pharmaceutical Compositions Thereof		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

I, Iwao OJIMA, declare the following:

1. My curriculum vitae is attached.

2. As described in the examples of the above-referenced patent application, omega-3 fatty acid – second-generation taxoid conjugates were successfully synthesized and evaluated for their *in vivo* anti-tumor activities against the drug-resistant pgp(+) human colon tumor xenografts DLD-1 and the drug-sensitive human ovarian tumor xenograft A121 in SCID mice. In the experiments using the paclitaxel-resistant, pgp(+) DLD1 human colon tumor xenograft implanted s.c. in SCID mice, paclitaxel and DHA-paclitaxel (Taxoprexin®) were totally ineffective. In sharp contrast, DHA-SB-T-1214 caused complete regression (CR) of the DLD-1 tumor in 5 of 5 mice at the 80 mg/kg dose administered on days 5, 8, and 11 (240 mg/kg total) (tumor growth delay >187 days). Figure A, on page 6 of the instant declaration, illustrates these results in graph form.

3. The *in vivo* results shown in the instant patent application are remarkable. These results could not have been predicted from the disclosure of the first generation DHA-taxane conjugates in U.S. 5,795,909. In particular, it is critical to note that U.S. 5,795,909 simply discloses *in vitro* cytotoxicity evaluations. A skilled artisan would have believed that the *in vivo* efficacy of DHA-taxoids could not have been predicted from such *in vitro* cytotoxicity evaluations, and hence would not have attributed much weight to these evaluations.

4. In the instant declaration, I provide additional comparative study results on the *in vivo* efficacy of a second-generation polyunsaturated fatty acid (PUFA)-taxoid conjugate, DHA-SB-T-1214, and DHA-paclitaxel (Taxoprexin®)/paclitaxel. These results further demonstrate the unexpected far greater superiority of DHA-SB-T-1214 over DHA-paclitaxel (Taxoprexin®) and paclitaxel.

5. Additional *in vivo* activity evaluation 1 (Experiment SB0409).

This experiment compared the efficacy of DHA-SBT-1214, paclitaxel and DHA-paclitaxel (Taxoprexin®) against a tumor model, the H460 human lung tumor xenograft implanted s.c. in SCID female mice using the q3dx3 schedule (day 5, 8 and 11). As Figure 1 and Table 1 show (on pages 7 and 8 of instant declaration), the results are consistent with my earlier findings on the distinctly superior efficacy of DHA-SB-T-1214 over paclitaxel and DHA-paclitaxel against multidrug resistant colon tumor xeongraft DLD-1. As the graph and table clearly indicate, DHA-SBT-1214 resulted in significant tumor growth delays or regression/eradication, depending on the dosage, as compared to paclitaxel or DHA-paclitaxel. All mice treated by paclitaxel and DHA-paclitaxel at the optimal doses died due to the aggressive tumor growth within 15 days after the first injection. In sharp contrast, mice treated by DHA-SB-T-1214 (@80 mg/kg/injection x 3) are all alive (6 in 6) without tumor on day 49 of the experiment. Since there was one toxic death with q3dx3 regiment at 80 mg/kg/dose (240 mg/kg total), the maximum tolerated dose (MTD) should be slightly lower dose. Thus, either 60 mg/kg/dose x 3 (180 mg/kg/injection) or 80 mg/kg/dose followed by 40 mg/dose x 2 (160 mg/kg total) may be the optimal dose. The results unambiguously demonstrate the distinct superiority of the second-generation DHA-taxane conjugate to the first generation DHA-taxane conjugate,

DHA-paclitaxel (Taxoprexin®). Also, it should be noted that DHA-paclitaxel (Taxoprexin®) and paclitaxel exhibit almost the same efficacy against this drug-resistant tumor xenograft, which is in very good agreement with the observation against another drug-resistant tumor xenograft, DLD-1 (human colon), as already presented.

6. Additional *in vivo* efficacy evaluation 2 (Experiment SB0209).

This experiment compared the *in vivo* efficacy of DHA-SB-T-1214 with that of paclitaxel against the PANC-1 human pancreatic tumor xenograft in SCID mice using the q3dx3 (day 5, 8, 11) and q7dx3 (day 5, 12, 19) regimens. Since paclitaxel and DHA-paclitaxel (Taxoprexin®) have exhibited practically indistinguishable efficacy against drug-resistant tumor xenografts, DLD-1 (colon) and HT460 (lung), my colleagues and I used only paclitaxel in this comparative study against another drug-resistant tumor xenograft, PANC-1. The results clearly indicate that DHA-SB-T-1214 were highly effective against this human pancreatic tumor xenograft with both q3dx3 and q7dx3 regimens. The MTD for DHA-SBT-1214 appears to be around 240 mg/kg total dosage (80 mg/kg/injection x 3) with one toxic death occurring at the 300 mg/kg total dosage. All mice that received DHA-SBT-1214 achieved tumor growth regressions, complete responses (CRs) and were essentially all cured. In stark contrast, paclitaxel was much less effective by comparison, achieving tumor growth regressions of only 18 days using the weekly schedule and only 13 days with q3dx3 schedule and no complete regressions. See Figure 2 and Table 2 on pages 9 and 10 of instant declaration.

7. Omega-3 polyunsaturated fatty acids such as α-linolenic acid (LNA) also exhibit remarkable efficacy *in vivo*.

My colleagues and I also investigated the use of different polyunsaturated fatty acids and their efficacies. My colleagues and I synthesized the conjugates of SB-T-1213 with DHA, LNA and LA (linoleic acid), and examined their efficacies against DLD-1 colon tumor xenograft (pgp^+) in the same manner as described above. As Figure 3 and Table 3 show (on pages 11 and 12 of instant declaration), LA-SB-T-1213 and LNA-SB-T-1213 exhibited strong antitumor

activity, while paclitaxel is ineffective. LNA-SB-T-1213 exhibited the complete regression in 2 of 5 mice tested against drug-resistant human colon tumor xenografts (Pgp+) DLD-1 (tumor growth delay>109 days). Although the toxicity of LNA-SB-T-1213 to the animals was higher than DHA-SB-T-1213, LNA-SB-T-1213 exhibited better overall activity than DHA-SB-T-1213 at the dose examined (which was not optimized). LA-SB-T-1213 did not show meaningful efficacy in the same assay, which revealed the marked difference between omega-3 fatty acids (LNA, DHA) and omega-6 fatty acid (LA). These results clearly indicate that omega-3 fatty acids other than DHA possess comparable efficacy as taxoid conjugates.

8. Experimental details for the *in vivo* efficacy evaluations (including the experiments described in the patent application)

Animals and tumor xenografts: Female severe combined immuno-deficient, (SCID) mice aged six to eight weeks were obtained from either the in-house breeding facility at Roswell Park Cancer Institute or the NCI breeding program. Various human tumor cell lines including A121 ovarian carcinoma, tumor DLD-1 colon tumor, PANC-1 pancreatic tumor and H460 non-small cell lung (NSCL) carcinoma were used. Tumors were initiated by implantation of approximately 50 mg of non-necrotic tumor fragments on the right flank. Chemotherapy was started when the tumor was established as a palpable mass (approximately 50-100 mm^3 size). Each drug treatment group or drug-free vehicle consisted of at least 4-5 mice per group, wherein untreated controls contained 10 mice per group.

Drug preparation for *in vivo* experiments: Paclitaxel or DHA-paclitaxel (Taxoprexin®) was prepared as a 7.5 mg/mL stock solution in equal parts of Cremophor ELP (BASF, Ludwigshafen, Germany) and absolute ethanol. These were used for comparison purposes (formulation with Cremophor/ethanol is standard for paclitaxel as well as DHA-paclitaxel). DHA-taxoids and other PUFA-taxoids were prepared either as a 30 mg/mL stock solution in equal parts of Tween 80 (polyoxyethylene-sorbitan monooleate; purchased from Sigma Chemical Company) and absolute ethanol (formulation with Tween 80/ethanol is standard for docetaxel) or Solutol. DHA-SBT-1214 was formulated by Chem Master International, Inc. at 50 mg/mL in Solutol HS-15 / EtOH (1:1), with *L*-ascorbic acid (3.9mM) and α-tocopherol (2.0 mM). To stabilize the

formulation of the DHA-taxoids and other PUFA-taxoids, antioxidants, *L*-ascorbic acid (3.9 mM) and α-tocopherol (2.0 mM), were added. Each stock solution was further diluted before use with 0.9% NaCl (saline) so that the appropriate concentration of each drug could be injected i.v. via the tail vein, in a volume of approximately 0.4 mL per 20 g mouse. Each drug was administered once a day, using either a q7dx3 or q3dx3 schedule, when the tumor mass was 50-100 mm^3.

In vivo tumor growth assay: For each animal, the tumor length (l) and width (w), each in mm, was measured using electronic calipers and recorded every 3-4 days. Tumor volume (v), in mm^3, was calculated using the formula: $v = 0.4(l \times w^2)$. The time in days to the pre-determined target tumor volume of 600 mm^3 was linearly interpolated from a plot of log (volume) versus time. Statistically significant differences in tumor volumes between control and drug-treated mice were determined by the Cox-Mantel test. For the test, the time-to-event data for animals that did not reach the target tumor volume, either because of long-term cure (defined as those animals that were still alive at the conclusion of the experiment whose tumors either completely regressed or did not reach the pre-set target volume) or early death due to drug toxicity, were treated as censored data. All statistical tests were two-sided.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. Further that these statements were made with the knowledge that willfully false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willfully false statements may jeopardize the validity of the application of any patent issued thereon.

Respectfully submitted,

Dated: March 9, 2010

Iwao Ojima

Figure A. Anti-tumor effect of DHA-taxoids delivered i.v. to SCID mice bearing a Pgp(+) DLD-1 human colon tumor xenograft (up to the day 201)

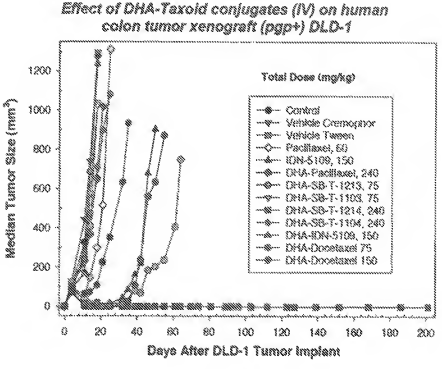

Figure 1. Exp. SB0409: *In vivo* efficacy of DHA-SBT-1214 compared with paclitaxel and DHA-paclitaxel (Taxoprexin®) in SCID mice implanted with H460 human lung carcinoma xenograft

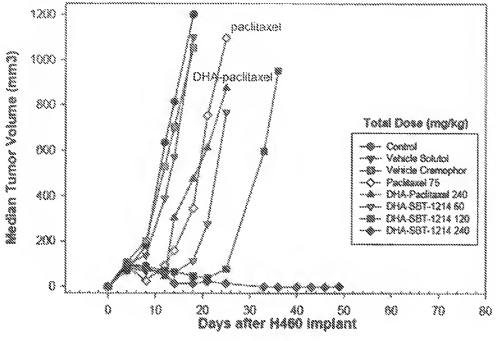

Table 1. Exp. SB0409: *In vivo* efficacy of DHA-SBT-1214 compared with paclitaxel and DHA-paclitaxel (Taxoprexin®) in SCID mice implanted with H460 human lung carcinoma xenograft

Treatment[a] q3dx3, i.v.	Total Dose (mg/kg)	Dose/inj (mg/kg)	Days to 600mm^3 Median (range)	*P*value[b] (Control)	Growth Delay (days)	Toxicity[c]	Tumor <600mm^3 /group[d]
Control	0	0	12 (12-20)	---	---	0 / 10	0 / 10
Vehicle : Solutol HS-15	-	-	14 (12-16)	.967	2	0 / 5	0 / 5
Vehicle: Cremophor	-	-	14 (12-16)	.833	2	0 / 5	0 / 5
Paclitaxel	75	25	20 (15-25)	.003	8	0 / 7	0 / 7
DHA-Paclitaxel	240	80	15 (12-32)	.039	3	0 / 6	0 / 6
DHA-SBT-1214	60	20	24 (17-32)	<.001	12	0 / 8	0 / 8
DHA-SBT-1214	120	40	33 (32-46)	<.001	21	0 / 6	1 / 6
DHA-SBT-1214	240	80	>49	<.001	>38	3/7(1td)	6 / 6

[a] Treatment of female SCID mice from dlar, on day 5, 8 and 11 after SC H460 human NSCLC implant
[b] Based on comparison of each group vs. control using the Cox-Mantel Test
[c] Number of animals who either died or lost greater than 20% initial body weight, td=toxic death
[d] Mice with tumor volume less than target size of 600mm^3 / # toxic death/ total animals; on Day 49

Figure 2. Exp. SB0209. *In vivo* efficacy of DHA-SBT-1214 compared with paclitaxel in SCID mice implanted with Panc-1 pancreatic carcinoma xenograft

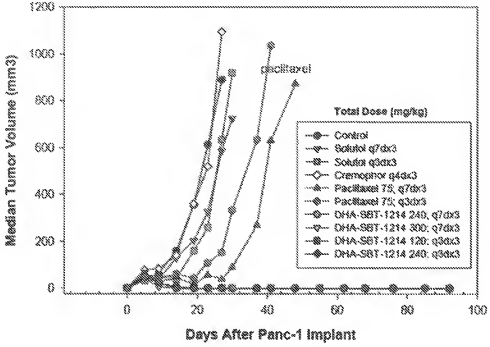

Table 2. Exp. SB0209: *In vivo* efficacy of DHA-SBT-1214 compared with paclitaxel in SCID mice implanted with Panc-1 pancreatic carcinoma xenograft

Treatment[a] *i.v.*	Total dose (mg/kg)	Dose/inj (mg/kg)	Days to 600mm^3 median (range)	*P*value[b] (Control)	Growth Delay (days)	Toxicity[c]	Tumor-free mice /group[d]
Control	0	0	23 (16-34)	---	---	0 / 10	0 / 10
Vehicle: q7dx3 Solutol HS-15	-	-	26 (23-32)	.780	3	0 / 4	0 / 4
Vehicle: q3dx3 Solutol HS-15	-	-	26 (23-31)	.827	3	0 / 4	0 / 4
Vehicle: q3dx3 Cremophor	-	-	24 (23-32)	.752	1	0 / 3	0 / 3
Paclitaxel q7dx3	75	25	41 (36-90+)	<.001	18	0 / 5	1 / 5
Paclitaxel q3dx3	75	25	36 (33-50)	.001	13	0 / 5	0 / 5
DHA-SBT-1214 q7dx3	240	80	>90	<.001	>71	0 / 6	6 / 6
DHA-SBT-1214 q7dx3	300	100	>90	<.001	>71	3/7 (1td)	6 / 6
DHA-SBT-1214 q3dx3	120	40	>90	<.001	>79	0 / 7	7 / 7
DHA-SBT-1214 q3dx3	240	80	>90	<.001	>79	0 / 7	7 / 7
SBT-1214 q3dx3	60	20	>90	<.001	>79	0 / 7	7 / 7
SBT-1214 q3dx3	120	40	>90	<.001	>79	0 / 7	7 / 7

[a] Treatment of female SCID mice from diar. on day 5, 8 and 11 after SC H460 human NSCLC implant
[b] Based on comparison of each group vs. control using the Cox-Mantel Test.
[c] Number of animals who either died or lost greater than 20% initial body weight, td = toxic death.
[d] Mice with no tumor/ total animals at end of experiment, Day 90.

Figure 3. Antitumor effect of PUFA-Taxoid conjugates delivered *i.v.* to SCID mice bearing a Pgp+ human colon tumor xenograft, DLD-1

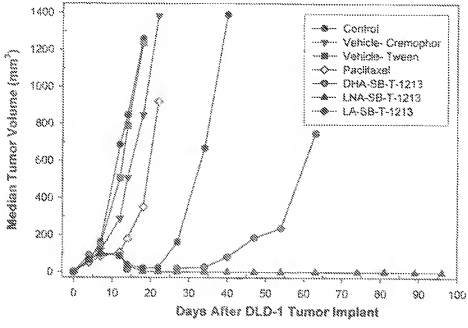

Table 3. Antitumor effect of PUFA-Taxoid conjugates delivered *i.v.* to SCID mice bearing a Pgp+ human colon tumor xenograft, DLD-1.

Treatment[a] (i.v.)	Total Dose (mg/kg)	Growth Delay (days)	Toxicity[b]	Cured mice[c]/ group
Control	0	---	0	0 / 7
Vehicle-Crem	0	---	0	0 / 4
Vehicle-Tween	0	---	0	0 / 4
Paclitaxel	75	9	0	1 / 5
DHA-SB-T-1213	75	54	0	0 / 5
LNA-SB-T-1213	75	>109	2	2 / 5
LA-SB-T-1213	75	21	1	0 / 5

[a]Treatment given *i.v.* to SCID mice on days 5, 8 and 11 after DLD-1 human colon tumor implant. Paclitaxel formulated in Cremophor : EtOH; DHA-taxoid conjugate, LNA-taxoid conjugate and LA-taxoid conjugate formulated in Tween:EtOH.
[b]Number of animals who either died or lost greater than 20% body weight.
[c]SCID mice with no palpable tumor on day 120, end of experiment.